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Keratinophilic fungal diversity of soil from Ernakulam and Thrissur districts - Kerala

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ABSTRACT

Soil is a well-known source of wide variety of microorganisms. This article briefly explains the isolation and identification of ecologically important keratinophilic fungi which are involved in the degradation of most abundant and highly stable animal protein – keratin. Soil is the home of several such fungi which are not even noticed. During the course of study approximately 15 different fungal species were isolated and identified. The isolation technique adopted was Vanbreuseghem's hair baiting .This humble attempt will create interest among the students to know more about the fungal diversity of soil and their enormous potentials. Trychophyton, Microsporum, Chrysosporium, Aspergillus etc. are some among the isolates.

Key words: kerainophilic fungus, keratin, hair baiting, keratinase

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INTRODUCTION

Fungi are an important component of the soil microbiota more in abundance than bacteria, depending on soil depth and nutrient conditions. The soil samples were collected from different sites of Ernakulam and Trichur districts. The soil collection mainly focussed the proximities of poultry farms, dumping sites of animal hair, hoof, nail etc. Different soils have specific fungus flora, but the majority of species found in them are cosmopolitan, (Ainsworth & Sussman, 1968). Fungi present in the soil were keratinophilic (keratin loving) and some keratinolytic (keratin digesting). Many of them are potential pathogen to both human and animals. Soil that are rich in keratinous materials are the most conducive for the growth and occurrence of keratinophilic fungi (Moallaei & Zaini, 2006). Keratinolytic mycoflora love to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof, beak etc. They utilize keratin as carbon source (Cooke, 1980). In general, the qualitative and quantitative composition of Keratinophilic fungi can be multifunctional bioindicator of environmental pollution with waste. It means that the composition indicates not only the presence of keratin reminants and feacal contaminants in the environment but also respond to the changes in environmental conditions.(Ulfig k, 2003 & Samuel. P,2011) Additionally, the fungal growth indices inform us about the infection risk associated with the contamination of the environment with potential fungal pathogensWater pollution is thought to reduce the diversity of sensitive fungi, while increasing the diversity of those that are less sensitive [Cooke 1970]. Keratinophilic fungi are important ecologically and present in the environment with variable distribution patterns and cause human and animal mycoses (Mohamed *et al*., 2000). This is a potential enzyme for removing hair and feather in the poultry industry Takami *et al.*, 1992, for nutritional upgrading of feather meal and conversion of feathers into a feed protein in feed industry(Williams et al.,1991) This paper reports the prevalence of keratinophilic fungi from the soil collected from different locations of Ernakulam and Trichur districts in Kerala.

MATERIALS AND METHODS

Sterilised hair strands, soil, distilled water, petridishes and Sabourand dextrose agar (SDA), milk etc. were needed for the study.

Preparation of SDA medium

Peptone	10gm
Dextrose	40gm
Agar	20gm
Milk	5gm/50ml distilled water
Streptomycin	1gm
Distilled water	1000ml.

Isolation of Keratinolytic Fungus

The keratinolytic nature of the fungus made it easy to isolate using Vanbreuseghem's hair bait technique initially developed by R. Vanbreuseghem, a Belgian mycologist in1952. Since then, a number of modifications have been developed, but the basic principle remains the same i.e use of natural keratin substrate as baits to recover the fungi from soil.

Collection of Soil: Soil was collected in sealed containers using sterile spoon from the habitats where keratin and hence keratinolytic fungi were present.

Hair Baiting:

1. Sterile petri plates were half filled with soil samples.
2. Short strands of sterilised hair were spread over the surface of soil.
3. About 10 to 15 ml. of water was added to the soil to facilitate the germination of the fungal spores. The bacterial growth was prevented by adding antibiotics.(Streptomycin)
4. The petri plates were kept in room temperature for 4-6 weeks for incubation.
5. The hair with fungus was carefully taken from the petri plates and transferred to another sterile test tube.
6. The tube with the hair was shaken vigorously with minor quantity of sterile saline.
7. The supernatant was carefully spread on milk agar (Sabourand dextrose agar (SDA + milk) medium.
8. After one week the colonies grown on the milk agar medium digesting the milk protein (examining the zone of clearance) were checked and the fungi were identified.

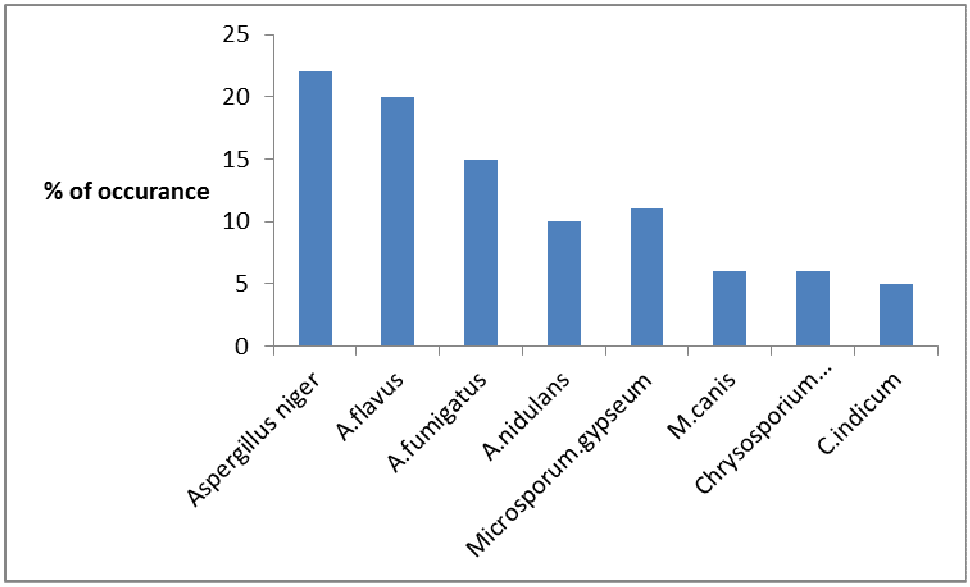
Identification Of Fungus

The isolated fungi were stained with Lactophenol cotton blue and observed it under the microscope. By noting the morphology of the fungus identification was done.

RESULTS AND DISCUSSION

Out of 60 soil samples collected from various locations of Ernakulam and Thrissur districts, 51 samples were found positive in fungal growth. A total of 5 genera and 15 species were isolated. In the study some of the soil samples gave single species and some yielded mixed growth of two or more species of fungi. Aspergillus species was isolated frequently. The other organisms obtained were *Microsporum, Chrysosporium, Trychophyton, Fusarium, Alternaria, Penicillium, Gymnascus* etc. Among the isolates *Aspergillus niger* dominated (22%) followed by *A.flavus* (20%) , *A.fumigatus* (15%) , *A.nidulans* (10%), *Microsporum gypseum* (11%), *M.canis* (6%), *Chrysosporium keratinophilum* (6%) *C.indicum* (5%), and the rest are *Trychophyton mentagrophytes, T. terrestre ,Gymnascus, Alternaria, Cladosporium, Paeciliomyces* and *Penicillium* .

No	Name of Fungus	% of Occurence
1	Aspergillus.niger	22
2	A.flavus	20
3	A.fumigatus	15
4	A.nidulans	10
5	Microsporum.gypseum	11
6	M.canis	6
7	Chrysosporium keratinophylum	6
8	C.indicum	5



Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. Keratinophilic fungi are important ecologically and recently have attracted attention throughout the world. They play a significant role in the natural degradation of keratinized residues (Sharma and Rajak, 2003; Fillipello et al., 1994; Fillipello, 2000) The keratinophilic fungi were found more in abundance in alkaline soil. *Aspergillums sps*. and *Chrysosporium* were frequently isolated from soil close to the poultry farm. This group of fungi are distributed worldwide. (Anbu *et al*., 2004).*A. niger* was the first dominant species(22%) followed by *A. flavus* isolated from the soil (20%). This species is present in soil present at different areas (Sarquis, 1990). The species also occurs in external ears and involved in otitis (Jesenka *et al*., 1992). On the other hand this species is a potential mycotoxin producer (Richardson, 2003). *A. fumigatus* causes skin, eye and ear infections (Bodey, 1989).

CONCLUSION

Among the microbes that cycle keratin protein in nature, keratinophilic fungi are very common and the most diverse. If keratinolytic fungi were not there to cycle this highly stable protein (keratin), we can imagine the quantity of keratin that would have accumulated on earth, since a vast quantity of keratin is shed by the vertebrates. Indian soils contain many more keratinophilic fungi than those presently recorded, and there is need for further taxonomic and ecological studies of this interesting group of organisms (Sharma R, Rajak RC (2003))The potential use of keratinases have different applications where keratins should be hydrolyzed, such as the leather and detergent industries, textiles, waste bioconversion, medicine, and cosmetics for drug delivery through nails and degradation of keratinized skin. Fungi also display lipolytic activity and remove petroleum hydrocarbons from the medium during degradation of proteins.

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